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**Widespread contamination of wildflower and bee-collected pollen
with complex mixtures of neonicotinoids and fungicides.**

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15 **Abstract**

16 There is considerable and ongoing debate as to the harm inflicted on bees by exposure to
17 agricultural pesticides. In part, the lack of consensus reflects a shortage of information on field-
18 realistic levels of exposure. Here, we quantify concentrations of neonicotinoid insecticides and
19 fungicides in the pollen of oilseed rape, and in pollen of wildflowers growing near arable fields. We
20 then compare this to concentrations of these pesticides found in pollen collected by honey bees and
21 in pollen and adult bees sampled from bumblebee colonies placed on arable farms. We also
22 compared this with levels found in bumblebee colonies placed in urban areas. Pollen of oilseed rape
23 was heavily contaminated with a broad range of pesticides, as was the pollen of wildflowers growing
24 nearby. Consequently, pollen collected by both bee species also contained a wide range of
25 pesticides, notably including the fungicides carbendazim, boscalid, flusilazole, metconazole,
26 tebuconazole and trifloxystrobin and the neonicotinoids thiamethoxam, thiacloprid and
27 imidacloprid. In bumblebees, fungicides carbendazim, boscalid, tebuconazole, flusilazole and
28 metconazole were present at concentrations up to 73 nanogram/gram (ng/g). Pesticide
29 concentrations in pollen collected by honeybees tended to be lower than those in pollen collected
30 by bumblebees. It is notable that pollen collected by bumblebees in rural areas contained high levels
31 of the neonicotinoids thiamethoxam (mean 18 ng/g) and thiacloprid (mean 2.9 ng/g), along with a
32 range of fungicides, some of which are known to act synergistically with neonicotinoids. Pesticide
33 exposure of bumblebee colonies in urban areas was much lower than in rural areas. Understanding
34 the effects of simultaneous exposure of bees to complex mixtures of pesticides remains a major
35 challenge.

36 **Keywords:** neonicotinoids, fungicides, pollen, bumblebees, honeybees

37 Introduction

38 The extent, causes and consequences of bee declines have attracted much scientific and public
39 attention in the last decade. It is clear that there is no single cause, but that several interacting
40 factors including declines in floral abundance and diversity resulting from agricultural intensification,
41 the spread of parasites and pathogens, and exposure to pesticides all contribute to these declines
42 (Goulson et al., 2015). The impact of pesticides, in particular the class of insecticides known as
43 neonicotinoids, on pollinator declines is the most controversial of these factors.

44 Neonicotinoids are neurotoxins which act as nicotinic acetylcholine receptor agonists in the central
45 nervous system of insects and cause overstimulation, paralysis, and death (Goulson 2013). These
46 pesticides are systemic and are widely applied as seed dressings to flowering crops, where they can
47 be detected at the low ng/g level in the nectar and pollen (Fairbrother et al., 2014). Pollen is a major
48 food source for growing larvae and nurse workers, and so is a likely source of exposure of bees to
49 neonicotinoids (Sanchez-Bayo and Goka 2014).

50 A key part of the debate over the impacts of neonicotinoids has become focussed on the dose that
51 bees are likely to be exposed to in the field. Laboratory and semi-field studies are often dismissed as
52 using unrealistically high doses of pesticides. For example Whitehorn et al. (2012) experimentally
53 exposed bumblebee colonies to pollen containing 6 ng/g of the neonicotinoid imidacloprid, plus 0.70
54 ng/g in their nectar, and found an 85% drop in queen production compared to controls. However, it
55 has since been argued that this dose was higher than bumblebees are likely to receive in the field
56 because colonies will be feeding on a mix of contaminated crops and uncontaminated wildflowers
57 (Carreck and Ratnieksi 2014). Thus obtaining more information on what constitutes field realistic
58 exposure to both bumblebee and honey bee colonies is vital to taking this debate forwards.

59 In addition to neonicotinoids, there is clear evidence that honey bees are routinely exposed to a
60 complex mixture of many different agrochemicals (Johnson et al., 2012). An analysis of honey bees
61 and their hive wax and pollen in the USA revealed that the majority of samples were contaminated
62 with at least one pesticide, and a total of 121 different agrochemicals, including metabolites and
63 miticides, were detected in samples (Mullin et al., 2010). Similarly 37 insecticide and fungicide
64 chemicals were detected in honey bees and hive products sampled in France (Lambert et al., 2013).
65 In addition to the active ingredients, bees may also be exposed to additives used in pesticide
66 formulations and these have also been detected in pollen and honey with the potential to interact
67 with pesticides and increase toxic effects (Mullin et al., 2015). Synergistic toxicity of some
68 combinations of insecticides and fungicides have been reported for honey bees or their larvae (Iwasa

et al., 2004; Schmuck et al., 2003; Thompson et al., 2014; Zhu et al., 2014). For example the toxicity of some neonicotinoids can be increased by as much as a factor of 1000 by simultaneous exposure to demethylation inhibiting (DMI) fungicides (Iwasa et al., 2004; Schmuck et al., 2003). DMI fungicides act by inhibiting Cytochrome P450 (CYP P450) mediated ergosterol biosynthesis in fungi and are thought to inhibit CYP P450 enzymes in insects that are important for detoxification of neonicotinoids and other insecticides (Schmuck et al., 2003).

Our study focusses on determining which mixtures of commonly used fungicides occur alongside neonicotinoids in crop and wildflower pollen and in the pollen collected by honey bees and bumblebees. Our aim is to investigate the potential for exposure of bees to mixtures of neonicotinoid and fungicide pesticides which are present in crop and wildflower pollen. Pesticides were analysed in pollen collected from oilseed rape (OSR) flowers, wildflowers growing in margins of OSR and winter wheat (WW) crops, and from pollen collected by honey bee (*Apis mellifera*) and bumblebee (*Bombus terrestris*) colonies placed in arable farmland. We also compare exposure of bumblebee nests placed in urban versus rural areas, and quantify residues in the adult bumblebees. Mixtures of a total of 20 agrochemicals were analysed comprising neonicotinoids and fungicides commonly used in UK crops.

2. Material and methods

2.1 Sample collection

2.1.1 Pollen collected from plants

- OSR pollen

Pollen samples from OSR flowers were collected in 7 fields from three farms located in East Sussex (United Kingdom) during the OSR blooming period (end of May – June 2013), and from 1 to 3 sites per OSR field were sampled (n=11 in total). To obtain pollen samples, OSR flowers were gathered, stored on ice in coolers in the field and then frozen immediately at -80°C until further handling. At processing, flower samples were gently defrosted and dried in an incubator at 37 °C for 24 hours to facilitate pollen release from the anthers. After drying, flowers were brushed over food strainers to separate pollen from anthers and sifted through multiple sieves of decreasing pore sizes (pore sizes from 250 to 45 µm).

- Wild plants in the field margins.

Wildflowers pollen samples were collected from 4 of the 7 OSR fields as well as in the margin of 4 WW fields present in same the 3 farms. Field boundaries in the region typically consist of a hedge of woody plants separated from the crop by a 0-2 m strip of herbaceous vegetation. The average sample distance from the crop edge was 1.5 m (range 1-2 m). Samples of pollen were collected from the wildflowers present in the field margins and hedge using the method described above for OSR plants. The species of wildflowers collected depended upon which species were available. In OSR field margins, pollen from 8 wildflower samples comprising 4 different species (*Ranunculus repens*, *Silene latifolia* (sampled 3 times), *Matricaria recutita* (x3), *Cirsium vulgare*) were collected. In WW margins, pollen from 13 wildflower samples comprising 8 different species (*Heracleum sphondylium* (x4), *Papaver rhoeas*, *Cirsium vulgare*, *Senecio jacobaea*, *Rosa canina*, *Pimpinella saxifraga*, *Aethusa cynapium* and *Matricaria recutita* (x3)) were collected. Pollen samples were analysed separately from each species with the exception of low amounts (< 20 mg) of four wildflower pollen samples collected from plants growing at the same site of a WW margin which were pooled and analysed as a single sample.

2.1.2 Pollen collected from bees.

- honey bees

Five honey bee (*Apis mellifera*) colonies were placed in the vicinity of the OSR fields at the beginning of the OSR flowering period (May 2013) and stayed in the same sites until the end of August 2013. Distances between the hives and the nearest OSR fields ranged from 1 to 260 m (see Table S1). The hives were equipped with pollen traps during 4 consecutive days at the beginning of June 2013 (i.e., during the OSR blooming period), and for 4 days in mid-August 2013 (i.e., when no OSR was in flower) in order to collect pollen loads from the returning honey bee foragers. After 4 days, the traps were removed from the hives and the pollen gathered and stored on ice in coolers in the field, and then at -80 °C until analysis. Pollen balls within each sample were sorted and weighed by colour (Human et al., 2013; Kirk 2006). Pollen grains associated with plant species were identified under a microscope following standard methods and using reference specimens and published reference collections (Demske et al., 2013; Moore et al., 1991; Sawyer 1981).

- bumblebees

Eight bumblebee nests (*Bombus terrestris audax*) were obtained from Agralan Ltd, Swindon, UK (originating from Biobest, Belgium). A sample of bumblebee workers from Biobest nests was analysed for target pesticides prior to the experiment and levels of all test analytes in bumblebee extracts were below the method detection limits. Five nests were placed in different farmland sites in South-East

England (East and West Sussex) at the beginning of May 2013. Sites were at least 1 km apart and in average 590 m far from the nearest OSR crop (range 8-1116 m, see Table S1). Three other nests were located in gardens from urban areas of West Sussex, being separated more than 4 km apart, and with an average distance to the nearest OSR crop of 1577 m (range 240-2670 m). After 4 weeks of free foraging in the field (comprising most of the OSR blooming period), pollen samples (> 200 mg) were collected from the in-nest stores in every colony using stainless steel micro-spoons, and were stored in 1.5 ml micro-centrifuge tubes at -80° C. Before extractions, every pollen sample was manually homogenised using a micro-spatula. A subsample of approximately 2 mg was evenly spread in a microscope slide, using glycerine jelly as the mounting medium. Light microscopy was used to identify the source of the pollen grains within the samples, and the proportion of the different taxa present in the samples was estimated by identifying pollen grains in five microscope fields of view uniformly distributed across the slide coverslip until 200 pollen grains were counted. After ten weeks of free foraging in the field, three to eight workers per nest were also collected for pesticide analysis of individual bees.

2.2 Pesticide analysis

2.2.1 Chemicals and reagents

Choice of analytes: Details of test analytes used in the study are given in Table 1. The pesticides comprised nine classes of contaminants and included all five of the neonicotinoid chemicals that are registered for use in the UK. Fungicides were chosen based on the most used (by weight) in UK crops including oilseed rape, wheat, spring barley, field bean, strawberry and raspberry crops (<https://secure.fera.defra.gov.uk/pusstats/surveys/2012surveys.cfm>). In addition, levels of an insecticide synergist piperonyl butoxide were also analysed as it is used in agrochemical formulations and has been reported to synergise the activity of some neonicotinoids (Bingham et al., 2008; Khan et al., 2015).

160 **Table 1. The list of chemicals analysed in this work, their chemical classes and their last applications in the studied oilseed rape (OSR) or winter wheat**
 161 **(WW) fields.**

Chemicals	Class	Last application		Application		Comments
		OSR field		WW field		
		Month	Year	Month	Year	
Insecticides						
Thiamethoxam	Neonicotinoid	Aug	2012	Aug	2011	seed dressing
Clothianidin	Neonicotinoid	March	2012	Oct	2012	seed dressing
Imidacloprid	Neonicotinoid	Not used				used prior to 2011
Acetamiprid	Neonicotinoid	Not used				used for gardening
Thiacloprid	Neonicotinoid	Not used				used in neighbouring fields in 2011 and 2012 and in gardens
Fungicides						
Carbendazim	Methyl benzimidazole carbamates (MBC)	May	2013	April	2012	spray
Carboxin	Succinate dehydrogenase inhibitors (SDI)	Not used				commonly used for barley crops ^a
Boscalid	Succinate dehydrogenase inhibitors	May	2013	May	2013	spray
Spiroxamine	Amines ("Morpholines") (SBI: Class II)	April	2012	June	2013	spray
Silthiofam	Thiophene	Not used				commonly used for WW ^a
Triticonazole	Demethylation inhibitors (DMI) (SBI: Class I)*			March	2011	spray
Epoxiconazole	Demethylation inhibitors (SBI: Class I)	April	2012	May	2013	spray
Tebuconazole	Demethylation inhibitors (SBI: Class I)	June	2012	June	2013	spray
Flusilazole	Demethylation inhibitors (SBI: Class I)	Jan	2013	Nov	2011	spray
Prochloraz	Demethylation inhibitors (SBI: Class I)			March	2011	spray
Metconazole	Demethylation inhibitors (SBI: Class I)	May	2013	Jan	2012	spray
Pyraclostrobin	Quinone outside inhibitors (QoI)	April	2012	May	2013	spray
Fluoxastrobin	Quinone outside inhibitors	May	2011	May	2011	spray
Trifloxystrobin	Quinone outside inhibitors			May	2011	spray
Synergist						
Piperonyl butoxide						used in the formulation of insecticides

162 ^a information from Defra report <https://secure.fera.defra.gov.uk/pusstats/surveys/2012surveys.cfm>.

163 * SBI = sterol biosynthesis inhibitor also known as Ergosterol biosynthesis inhibitor (EBI) - an inhibitor of sterol synthesis, which is essential for fungal growth. EBI
 164 fungicides include DMIs as well as the morpholines and piperidines.

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Certified standards of carbendazim, thiamethoxam, thiamethoxam-d₃, clothianidin, clothianidin-d₃, imidacloprid, imidacloprid-d₄, acetamiprid, thiacloprid, carboxin, boscalid, spiromamine, silthiofam, triticonazole, epoxiconazole, tebuconazole, flusilazole, prochloraz, metconazole, pyraclostrobin, trifloxystrobin, fluoxastrobin, piperonyl butoxide and also formic acid, ammonium formate, magnesium sulphate, sodium acetate and SupelTM QuE PSA/C18/GCB (ratio 1/1/1) were obtained from Sigma Aldrich UK. Certified standards of carbendazim-d₃ and tebuconazole-d₆ were purchased from LGC standards UK and prochloraz-d₇ and carbamazepine-d₁₀ from QMX Laboratories Limited UK. All pesticide standards were > 99% compound purity (except triticonazole: 98.8%, spiromamine: 98.5% and piperonyl butoxide: 97.9%) and deuterated standards > 97% isotopic purity. HPLC grade acetonitrile, toluene, methanol and water were obtained from Rathburns UK. Individual standard pesticide (native and deuterated) stock solutions (1 mg/ml) were prepared in acetonitrile (ACN) as was an internal standard mixture of the seven deuterated pesticides at 100 ng/ml. Calibration points in H₂O:ACN (70:30) were prepared weekly from the stock solutions. All solutions were stored at -20°C in the dark.

2.2.2 Sample preparation for neonicotinoid analyses

- *Pollen samples*

Pollen samples were extracted as described in David et al. (2015). Briefly, 100 mg (\pm 5 mg) of pollen sample was weighed and 400 μ g of the mix of deuterated internal standards in ACN were added to each sample which was then extracted using a modified QuEChERS method. First, 400 μ l of water was added and samples were then extracted by adding 500 μ l of ACN and mixing on a multi axis rotator for 10 min. Then, 250 mg of magnesium sulphate: sodium acetate mix (4:1) was added to each tube. After centrifugation (13,000 RCF for 5 min), the supernatant was removed into a clean Eppendorf tube containing 50 mg of SupelTM QuE PSA/C18/GCB and vortexed (10 s). The extract was mixed on a multi-axis rotator (10 min) and then centrifuged (10 min). The supernatant was transferred into a glass tube. The PSA/C18/GCB phase was then extracted with ACN/toluene (3/1, 150 μ l vortex 15 s). After centrifugation, the supernatant was combined with that of the previous ACN extract and spin filtered (0.22 μ m). The extract was evaporated to dryness under vacuum, and finally reconstituted with 120 μ l ACN:H₂O (30:70). Finally, the extract was centrifuged for 20 min and the supernatant stored at -20°C in the dark until analysis.

- *Bumblebee samples*

Bumblebees were first checked for adhering pollen residues in order to remove them before analysis. Individual whole bumblebee samples were ground in liquid nitrogen with a pestle and mortar followed

by manual homogenisation using a micro-spatula. Each bumblebee sample was then accurately weighed (average weight \pm standard deviation was 123 ± 83 mg). Then, 400 μ l of water was added and the samples were homogenised for 20 s using a vortex. Samples were then extracted using the same modified QuEChERS method as above (i.e, 500 μ l of ACN, 250 of magnesium sulphate: sodium acetate mix (4:1) and 50 mg of PSA/C18/GCB). Extracts were reconstituted, centrifuged and stored as above.

2.2.3 UHPLC-MS/MS analyses

The UHPLC-MS/MS method described in David et al. (2015) was used for the analysis of samples. Briefly, sample extracts were analysed using a Waters Acquity UHPLC system coupled to a Quattro Premier triple quadrupole mass spectrometer from Micromass (Waters, Manchester, UK). Pesticides in extracts were separated using a reverse phase Acquity UHPLC BEH C18 column (1.7 μ m, 2.1 mm \times 100 mm, Waters, Manchester, UK) fitted with a ACQUITY UHPLC BEH C18 VanGuard pre-column (130 \AA , 1.7 μ m, 2.1 mm \times 5 mm, Waters, Manchester, UK) and maintained at 22 $^{\circ}$ C. Injection volume was 20 μ l and mobile phase solvents were 95% water, 5% ACN, 5 mM ammonium formate, 0.1% formic acid (A) and 95% ACN, 5% water, 5 mM ammonium formate, 0.1% formic acid (B). Methods were developed to separate all 20 test analytes within a 25 min run. The initial ratio (A:B) was 90:10 and separation was achieved at 22 $^{\circ}$ C using a flow rate of 0.15 ml/min with the following gradient: 90:10 to 70:30 in 10 min; from 70:30 to 45:55 at 11 min, from 45:55 to 43:57 at 20 min, from 43:57 to 0:100 at 22 min and held for 8 min prior to return to initial conditions and equilibration for 5 min.

MS/MS was performed in the multiple reaction monitoring (MRM) using ESI in the positive mode and two characteristic fragmentations of the deprotonated molecular ion $[M+H]^+$ were monitored for quantification and confirmation (David et al., 2015). Argon was used as collision gas (P collision cell: 3×10^{-3} mbar), and nitrogen was used as desolvation gas (600 L/h). Mass calibration of the spectrometer was performed with sodium iodide. Data were acquired using MassLynx 4.1 and the quantification was carried out by calculating the response factor of neonicotinoid and fungicide compounds to their respective internal standards. Analyte concentrations were determined using a least-square linear regression analysis of the peak area ratio versus the concentration ratio (native analyte to deuterated IS). A minimum of six point calibration curves ($R^2 > 0.99$) were used to cover the range of concentrations observed in the different matrices for all compounds, within the linear range of the instrument. Method detection limits (MDL) and method quantification limits (MQL) for pollen and bumblebee matrices are given in Table S2.

2.2.4 Quality control

One workup sample (i.e., using extraction methods without a pollen/bee sample) per batch was injected on the UHPLC-MS/MS at the beginning of the run to ensure that no contamination occurred during the sample preparation. Solvent samples (ACN:H₂O (30:70)) were also injected between sample batches to ensure that there was no carryover in the UHPLC system that might affect adjacent results in analytical runs. Identities of detected neonicotinoids and fungicides were confirmed by comparing ratios of MRM transitions in samples and pure standards. The standard calibration mixture was injected before and after all sample batches to monitor sensitivity changes, and quality control samples (QCs, i.e., standard solutions) were injected every 10 samples to monitor the sensitivity changes during the analysis of each batch.

2.3 Statistical analysis

All statistical analyses were carried out using GraphPad Prism 6 software. Pesticide concentrations in the different pollen matrices were tested for normality using the D'Agostino-Pearson test. As pesticide concentrations were not normally distributed for many pesticides in the different pollen types, non-parametric Mann-Whitney U-tests were used to compare the concentrations of neonicotinoids and fungicides present in pollen collected from OSR flowers, wildflowers and honey bees, and for bumblebees and their pollen collected from urban and rural areas. To perform the statistical analyses, all concentrations that were over the limits of detection (\geq MDL) but below the limits of quantification ($<$ MQL) were assigned the value considered as the MDL in each case. Concentrations below the MDL were considered to be zero.

3. Results

3.1 Neonicotinoid and fungicide residues in pollen samples from oilseed rape, wildflowers from field margins and pollen collected by honey bees.

3.1.1 Frequencies, ranges and mean concentrations

Mixtures of neonicotinoids and fungicides were analysed in pollen samples from OSR flowers, wildflowers from OSR and WW margins and pollen collected by honey bees (during and after the OSR bloom) in order to estimate exposure of bees to these pesticides. All the different types of pollen were collected in each of the 3 different farms. Frequencies of each pesticide (i.e., percentage of samples with detectable levels of pesticides) as well as the ranges, mean and median concentrations found in the different pollens are presented in Table 2 (for raw data see Table S3 to S7).

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Table 2. The mean, median and range of concentrations and frequency of detection of neonicotinoid and fungicide chemicals in pollen collected from oilseed rape flowers, wild flowers and by honey bees during and after the OSR bloom.

	OSR pollen				Wildflower pollen								Honeybee pollen							
					OSR Margins				WW Margins				During OSR bloom				After OSR bloom			
	n = 11				n = 8				n = 10				n = 25				n = 19			
	Freq %	Range ppb	Mean ppb	Median ppb	Freq %	Range ppb	Mean ppb	Median ppb	Freq %	Range ppb	Mean ppb	Median ppb	Freq %	Range ppb	Mean ppb	Median ppb	Freq %	Range ppb	Mean ppb	Median ppb
Thiamethoxam	100	2.4 - 11	5.7	3.9	50	<0.12 - 21	2.8	<0.36	30	<0.12 - 0.50	0.13	<0.12	60	<0.12 - 1.6	0.15	<0.36	21	<0.12 - <0.36		
Clothianidin	73	<0.72 - 11	3.6	3.8	0	<0.72			10	<0.72 - 5.0	0.50	<0.72	8	<0.72 - <2.2			0	<0.72		
Imidacloprid	0	<0.36			13	<0.36 - <1.1			0	<0.36			12	<0.36 - 3.5	0.20	<0.36	5	<0.36 - <1.1		
Acetamiprid	0	<0.02			0	<0.02			0	<0.02			4	<0.02 - <0.07			0	<0.02		
Thiacloprid	100	<0.22 - 78	19	7.5	63	<0.07 - 4.0	0.60	<0.22	20	<0.07 - 2.9	0.30	<0.07	48	<0.07 - 10	0.90	<0.07	0	<0.07		
Carbendazim	100	0.60 - 163	39	13	100	1.3 - 6.8	3.5	3.5	0	<0.08			96	<0.08 - 120	12	2.5	74	<0.08 - 1.4	0.40	0.34
Carboxin	0	<0.12			0	<0.12			0	<0.12			0	<0.12			0	<0.12		
Boscalid	18	<0.12 - 25	3.2	<0.12	63	<0.12 - 38	5.8	0.53	60	<0.12 - 38	8.5	1.7	52	<0.12 - 21	5.2	<0.36	37	<0.12 - 17	2.5	<0.12
Spiroxamine	100	13 - 328	80	58	88	<0.02 - 151	47	7.3	70	<0.02 - 26	7.7	6.3	28	<0.02 - 74	3.4	<0.02	47	<0.02 - 1.1	0.20	<0.02
Silthiofam	0	<0.24			0	<0.24			0	<0.24			0	<0.24			0	<0.24		
Triticonazole	0	<0.24			0	<0.24			0	<0.24			0	<0.24			0	<0.24		
Epoxiconazole	64	<0.84 - 27	4.3	2.5	0	<0.84			0	<0.84			0	<0.84			5	<0.84 - 8.3	0.40	<0.84
Tebuconazole	100	1.5 - 21	5.2	2.9	75	<0.24 - 8.5	3.3	3.2	90	<0.24 - 34	7.0	3.2	76	<0.24 - 19	1.4	<0.72	79	<0.24 - 6.4	1.2	0.85
Flusilazole	18	<0.24 - 16	1.6	<0.24	25	<0.24 - 5.0	0.80	<0.24	0	<0.24			12	<0.24 - 6.1	0.30	<0.24	0	<0.24		
Prochloraz	0	<0.36			0	<0.36			0	<0.36			0	<0.36			0	<0.36		
Metconazole	27	<0.30 - 19	2.5	<0.30	0	<0.30			0	<0.30			12	<0.30 - 12	1.0	<0.30	0	<0.30		
Pyraclostrobin	9	<0.24 - 5.4	0.50	<0.24	38	<0.24 - 4.3	1.0	<0.24	10	<0.24 - 2.8	0.30	<0.24	28	<0.24 - 9.8	0.90	<0.24	16	<0.24 - 3.7	0.40	<0.24
Trifloxystrobin	45	<0.24 - 18	2.6	<0.24	63	<0.24 - 104	13	<0.72	20	<0.24 - 1.0	0.10	<0.24	40	<0.24 - 10	1.4	<0.24	16	<0.24 - 1.0	0.10	<0.24
Fluoxastrobin	18	<0.01 - <0.02			50	<0.01 - <0.02			30	<0.01 - <0.02			12	<0.01 - <0.02			11	<0.01 - 3.9	0.20	<0.01
Piperonyl butoxide	0	<0.72			0	<0.72			0	<0.72			0	<0.72			0	<0.72		

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Pollen traps were used to collect pollen brought back to honeybee hives (5) both during the OSR blooming period and later in the summer. Pollen was separated into wildflower species and analysed separately (n=3, 4, 5, 5 and 8 for hives 1, 2, 3, 4 and 5, respectively during the OSR bloom and n=5, 4, 2, 5 and 3 for hives 1, 2, 3, 4 and 5, respectively after the OSR bloom). ppb = ng/g wet weight of sample.

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266 - *OSR flowers*

267 As expected, the number of detected pesticides, their frequencies, their ranges as well as their mean
268 concentrations were generally higher in pollen from OSR flowers than in wildflower pollen and pollen
269 collected by honey bees (Table 2). All individual OSR pollen samples contained at least 6 neonicotinoid
270 and fungicide residues and most samples contained between 7 and 12 different pesticides.
271 Thiamethoxam, thiacloprid, carbendazim and spiroxamine were the most frequently detected
272 compounds (all present in 100% of samples), followed by tebuconazole (80%) clothianidin (73%),
273 epoxiconazole (64%) and trifloxystrobin (45%). The other fungicides (i.e., boscalid, flusilazole,
274 metconazole, pyraclostrobin and fluoxastrobin) were detected in less than 30% in these samples from
275 OSR flowers. Pesticides such as carbendazim and spiroxamine were present in some samples at
276 concentrations > 100 ng/g. The range of concentrations for other fungicides were between < MDL –
277 27 ng/g, and neonicotinoid concentrations were detected at between < MDL – 78 ng/g. With the
278 exception of thiacloprid which was only applied to neighbouring fields, thiamethoxam, clothianidin,
279 carbendazim, boscalid, spiroxamine, epoxiconazole, tebuconazole flusilazole, metconazole,
280 pyraclostrobin and fluoxastrobin had been applied in the studied OSR fields in the year of the sampling
281 or up to two years before the sampling (i.e., before the rotation to OSR crop). Trifloxystrobin had been
282 applied to WW fields present in the same farms two years before the sampling period (Table 1).

283 - *Wildflower pollen*

284 Pollen from four wildflower species was collected from 8 OSR field margins between June and August
285 2013. A similar mixture of pesticides as OSR pollen was detected in pollen from wildflowers growing
286 in the OSR field margins; however their frequencies of detection and concentration ranges were
287 generally lower than for OSR pollen (Table 2, Figure 1). Concentrations of thiamethoxam (Mann-
288 Whitney test, $U=11$, $p=0.0045$) and thiacloprid (Mann-Whitney test, $U=6$, $p=0.0006$) were significantly
289 lower in wildflower pollen compared with OSR pollen. Nevertheless, it is worth nothing that the
290 highest concentration of thiamethoxam were measured in the pollen from a wildflower (21 ng/g
291 detected in pollen from *Matricaria recutita* flowers growing in the margin from OSR field 2 in farm 2,
292 Table S4). Pollen was collected from 13 wildflower samples comprising 8 different species growing in
293 8 margins of WW fields between July and August. Three neonicotinoids and six fungicides were also
294 detected in wildflower pollen collected in WW field margins, and all the agrochemicals had been
295 applied previously to WW or to nearby fields. Concentrations of most pesticides were the same in
296 pollen samples collected from the wildflowers growing in WW and OSR field margins with the
297 exception of thiacloprid (Mann-Whitney test, $U=3$, $p=0.002$) which was lower in wildflower pollen
298 from WW field margins.

Pollen traps were used to collect pollen brought back to honey bee hives placed on the farms, both during the OSR blooming period (beginning of June 2013), and later in the summer when no OSR was in flower (mid-August 2013). Honeybee pollen balls were sorted by species in order to study the variability in exposure levels and sub-samples that were > 100 mg were analysed separately. (the pesticide concentrations for the composite samples brought to the hives were also calculated for later comparison with pollen samples collected from the bumblebee nests). During June 2013, the honey bee collected pollen included 9 wildflower species and OSR pollen, and 12 wildflower species in August, and the total pollen analysed comprised >86% of the total honey bee collected pollen in June and >75% of the total honey bee collected pollen in August (Tables S6 and S7). In terms of weight, the majority of these pollen samples collected by honey bees during the OSR flowering was from wildflowers, with just 10% of pollen coming from OSR (Botías et al., 2015). All pollen samples collected by honey bees were contaminated with a mixture of neonicotinoids and fungicides; a total of 14 compounds in pollen collected during OSR blooming and 10 after the bloom period. The number of pesticides found in any one pollen sample ranged between 2 to 8 compounds. A similar mixture of neonicotinoids and fungicides were detected in honey bee collected pollen in June as that present in wildflowers and OSR pollen, however, these compounds were at lower concentrations in honey bee corbicular pollen (Figure 1). The total concentrations of pesticides in honey bee pollen were lower in August compared with June and significantly reduced for carbendazim (Mann-Whitney test, $U=54$, $p<0.0001$) and thiamethoxam (Mann-Whitney test, $U=131.5$, $p=0.0047$). In addition, clothianidin, thiacloprid, flusilazole and metconazole were no longer detected in honey bee collected pollen at this time.

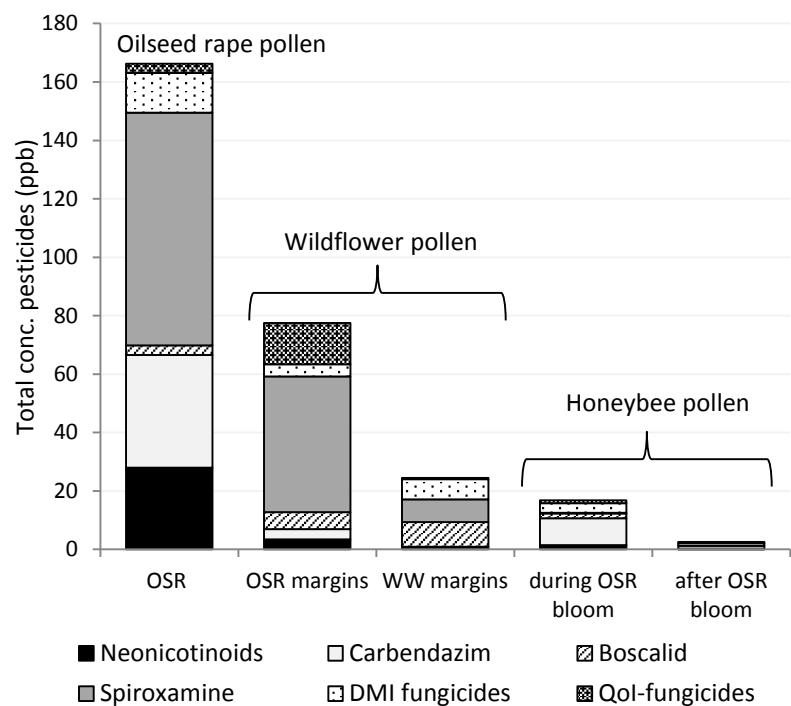


Figure 1. The sum of the mean concentrations of neonicotinoids and fungicides in pollen samples from oilseed rape (OSR) flowers (n=11), wildflowers from OSR margins (n=8) and WW margins (n=10), and collected by honeybees during OSR bloom (n=5) and after OSR bloom (n=5). OSR and wildflower pollens were collected in 3 farms, honeybee pollen samples were collected from hives sited on the vicinity of these farms. For the honeybee collected pollen, concentrations of the whole composite samples brought to the hives were used for the calculation of the means (i.e. one sample per hive was analysed).

Overall these results reveal that pollen collected by honey bees are contaminated by similar mixtures of pesticides as those present in wildflower pollen collected from OSR or WW field margins. The most frequently detected (>28%) pesticides both in honey bee collected pollen and wildflower pollen were thiamethoxam, thiacloprid, carbendazim, boscalid, spiroxamine, tebuconazole, pyraclostrobin and trifloxystrobin. Carbendazim and spiroxamine were detected at concentrations up to several hundreds of ng/g in some pollen samples. The totals for the mean measured concentrations of pesticides in pollen were 166 ng/g from OSR, and for wildflowers sampled from OSR and WW margins 78 and 25 ng/g respectively, and for honey bee pollen sampled during and after the OSR blooming period 17 and 2.6 ng/g respectively (concentrations of the whole composite pollen samples brought to the hives were used for the calculation of the means).

3.2 Neonicotinoid and fungicide levels in stored pollen and bumblebee individuals from nests placed in rural and urban areas

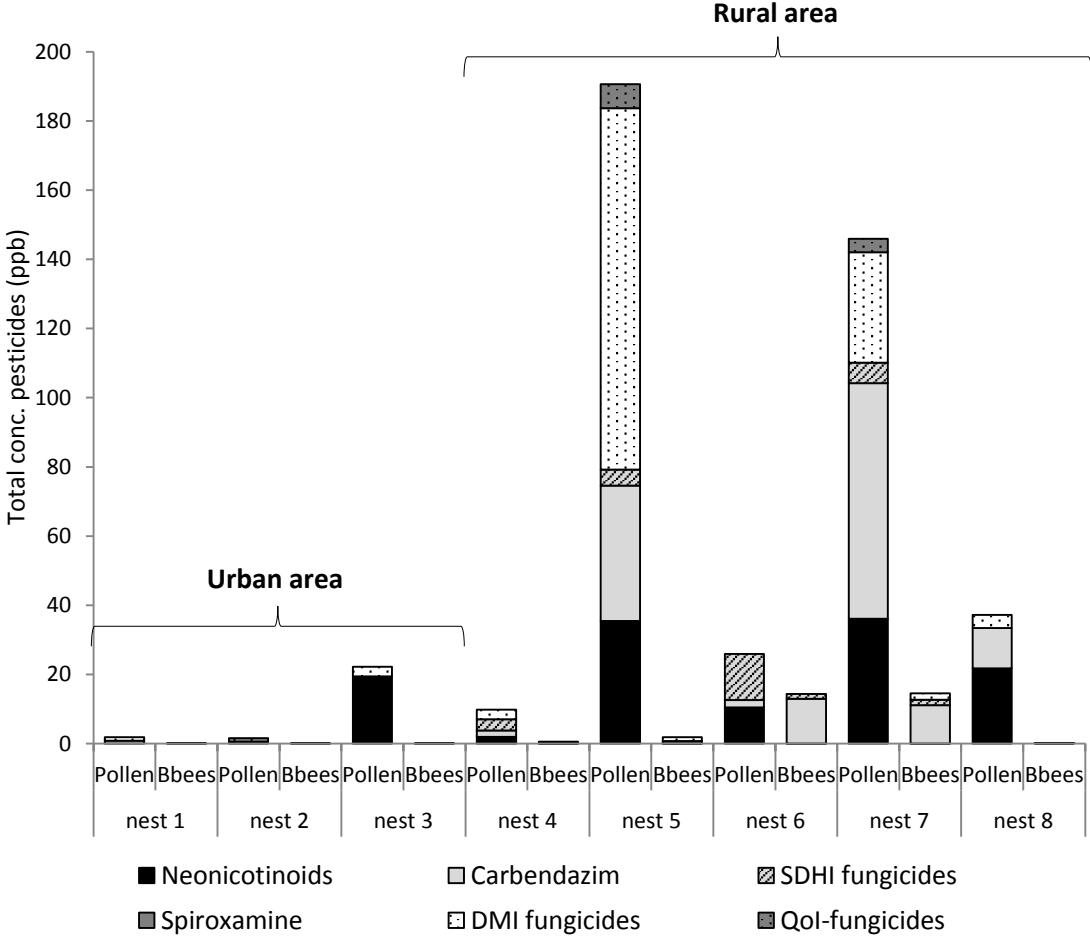
The presence of neonicotinoids and fungicide mixtures in pollen and individual bumblebees sampled from nests placed either in rural farmland or urban environments was determined. The range, mean and median of the pesticide levels found are presented in Table 3.

Pollen samples collected from the stores of individual nests placed in rural areas (n=5) contained between 3 to 10 pesticide compounds. The most frequently detected compounds (35-100%) included thiamethoxam, thiacloprid, carbendazim, boscalid, tebuconazole, flusilazole, metconazole and trifloxystrobin and at concentrations up to 68 ng/g for carbendazim and 84 ng/g for flusilazole. Imidacloprid, prochloraz and pyraclostrobin were also detected in 6% of the samples. Spiroxamine, although frequently detected at high concentrations in OSR and wildflower margin pollen, was below the MDL in bumblebee-collected pollen. The pollen from every nest was analysed as a whole, but the analysis of identity and proportion of pollen types under light microscopy revealed that it comprised a number of wildflower taxa with Rosaceae (*Crataegus monogyna*/*Malus* type) representing 42% in average of the visited plants, and 32% on average coming from OSR flowers (Table S9). In bumblebee individuals, the neonicotinoids thiamethoxam, acetamiprid and thiacloprid were detected at concentrations below their MQLs. Carbendazim (up to 73 ng/g), boscalid (up to 10 ng/g), tebuconazole (up to 5 ng/g), flusilazole and metconazole were detected above the MQLs in several individuals. Carbendazim, boscalid, tebuconazole, flusilazole and metconazole were the most frequently detected in 18-64% of individual bees. A comparison of the total pesticide concentrations in bumblebee and pollen samples revealed large differences in pesticide contamination and exposure between each nest (Figure 2).

365 **Table 3. The range, mean and median concentrations and frequency of detection of neonicotinoid and fungicide levels detected in stored pollen and in**
366 **individual bumblebees sampled from nests sited in rural and urban landscapes.**

	Rural area								Urban area							
	Bumblebee pollen				Bumblebee				Bumblebee pollen				Bumblebee			
	Freq %	Range <i>ppb</i>	Mean <i>ppb</i>	Median <i>ppb</i>	Freq %	Range <i>ppb</i>	Mean <i>ppb</i>	Median <i>ppb</i>	Freq %	Range <i>ppb</i>	Mean <i>ppb</i>	Median <i>ppb</i>	Freq %	Range <i>ppb</i>	Mean <i>ppb</i>	Median <i>ppb</i>
Thiamethoxam	100	1.7 - 35	18	21	7	<0.03 - <0.09			0	<0.12			7	<0.03 - <0.09		
Clothianidin	0	<0.72			0	<0.48			0	<0.72			0	<0.48		
Imidacloprid	20	<0.36 - <1.1			0	<0.72			33	<0.36 - 20	6.5	<0.36	0	<0.72		
Acetamiprid	0	<0.02			7	<0.01 - <0.04			33	<0.02 - <0.07			0	<0.01		
Thiacloprid	60	<0.07 - 13	2.9	0.45	18	<0.02 - <0.07			0	<0.07			40	<0.02 - 0.17	0.02	<0.02
Carbendazim	100	1.8- 68	24	12	64	<0.05 - 73	4.6	0.25	67	<0.08 - 0.80	0.40	0.36	0	<0.05		
Carboxin	0	<0.12			0	<0.24			0	<0.12			0	<0.24		
Boscalid	80	<0.12 - 13	5.4	4.6	36	<0.24 - 9.8	0.60	<0.24	0	<0.12			0	<0.24		
Spiroxamine	0	<0.02			0	<0.05			0	<0.02			0	<0.05		
Silthiofam	0	<0.24			0	<0.24			0	<0.24			0	<0.24		
Triticonazole	0	<0.24			0	<0.48			0	<0.24			0	<0.48		
Epoxiconazole	0	<0.84			0	<0.96			33	<0.84 - 2.8	0.90	<0.84	0	<0.96		
Tebuconazole	80	<0.24 - 15	4.1	2.8	18	<0.12 - 5.2	0.20	<0.12	67	<0.24 - 1.1	0.40	<0.72	7	<0.12 - <0.36		
Flusilazole	40	<0.24 - 84	17	<0.24	14	<0.12 - 1.9	0.20	<0.12	0	<0.24			0	<0.12		
Prochloraz	20	<0.36 - 11	2.2	<0.36	0	<0.36			0	<0.36			0	<0.30		
Metconazole	40	<0.30 - 19	4.3	<0.30	4	<0.24 - 1.1	0.04	<0.24	0	<0.30			0	<0.24		
Pyraclostrobin	20	<0.24 - 2.4	0.50	<0.24	0	<0.24			33	<0.24 - 1.0	0.30	<0.24	0	<0.24		
Trifloxystrobin	40	<0.24 - 4.4	1.7	<0.24	0	<0.01			0	<0.24			0	<0.01		
Fluoxastrobin	20	<0.01 -<0.02			0	<0.24			0	<0.01			0	<0.24		
Piperonyl butoxide	0	<0.72			0	<0.24			0	<0.72			0	<0.24		

367 Pollen and bumblebees were collected from the same nests. Between 5 and 8 individuals per nest were analysed (except for one nest where only 3 workers
368 were available). For the calculations of means and medians, all concentrations that were over the limits of detection (\geq MDL) but below the limits of
369 quantification ($<$ MQL) were assigned the MDL value, whilst concentrations below the MDL were considered to be zero. ppb = ng/g wet weight of sample.
370 Compounds highlighted in bold correspond to pesticides that were commonly found in pollen from both rural and urban areas.



374 **Figure 2. The sum of the mean concentrations of neonicotinoids and fungicides in individual bumblebees (bbees) and collected pollen in nests sited in**
375 **urban and rural areas.**

Concentrations of pesticides in pollen and bees sampled in urban areas (n=3) were much lower compared with rural areas (Figure 2). In nests placed in urban areas, six pesticides were detected in pollen collected by bumblebees; imidacloprid, acetamiprid, carbendazim, epoxiconazole, tebuconazole and pyraclostrobin. Imidacloprid was detected in pollen at up to 20 ng/g. Thiamethoxam, thiacloprid and tebuconazole were detected in bumblebee individuals at concentrations < 1 ng/g. Imidacloprid, carbendazim, tebuconazole and pyraclostrobin are the pesticides that were commonly found in pollen from both rural and urban areas.

A comparison of pollen collected by honey bees and bumblebees during the OSR bloom in rural landscapes revealed that many of the neonicotinoid and fungicide compounds which were present at concentrations > 1 ng/g were common to pollen collected by both bee species, but in this study exposure appeared to be much higher for bumblebees (Figure 3).

The insecticide synergist piperonyl butoxide was not detected in any of the pollen samples in this study.

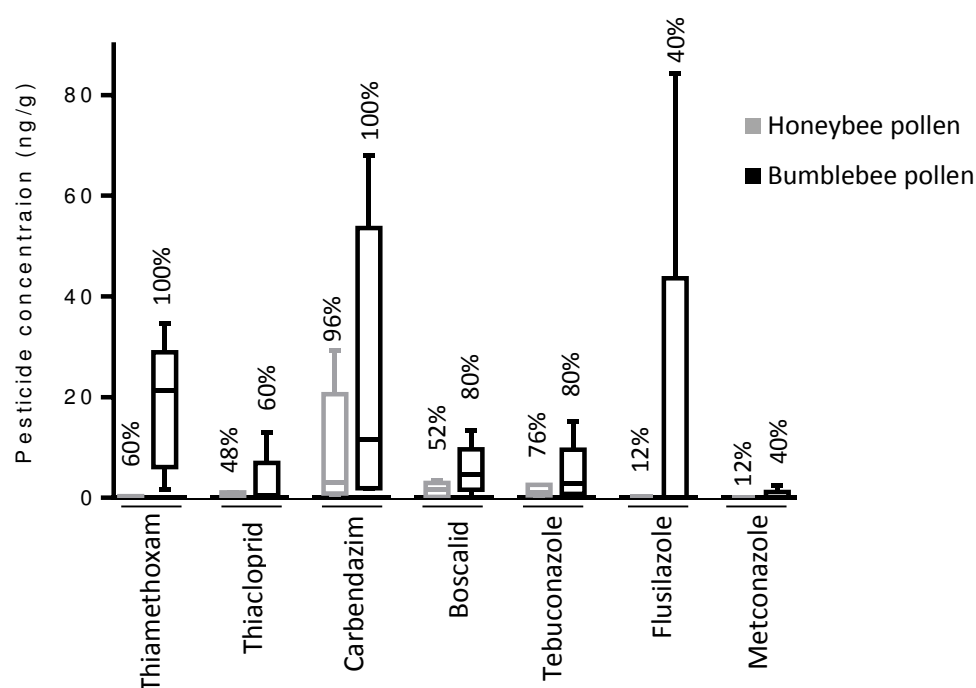


Figure 3. Levels of thiamethoxam, thiacloprid, carbendazim, boscalid, tebuconazole, flusilazole and metaconazole in pollen samples collected by honeybee (n=5 beehives) and bumblebees (n=5 nests). Honeybee hives were placed in farms near OSR fields and the pollen was collected during the OSR bloom for 4 days using pollen traps. Concentrations of the whole composite samples brought to the hives were used for the calculation of the means. Bumblebee nests were placed in rural areas in arable landscapes and the pollen was collected after 4 weeks of free foraging in the field. The frequency of detection of neonicotinoid and fungicide are indicated above each box-and-whiskers-plots. The length of each box corresponds to the interquartile range, the upper and lower boundary

of the box representing 75th and 25th percentiles, respectively. The upper and lower whiskers represent the maximum and the minimum values, respectively. The line in the box indicates the median value.

4. Discussion

Debates over the impacts of pesticides on bees have tended to focus on the effects of specific compounds or groups of compounds, with much attention in recent years on neonicotinoid insecticides. However, it has recently become clear that honeybees are chronically exposed to complex mixtures of pesticides (Johnson et al., 2012). Here, we show that both flowering crops and nearby wildflowers are contaminated with a broad range of pesticides, and that this translates into exposure of both honey bees and bumblebees to similar complex mixtures, with marked differences in concentrations of pesticides in pollen collected by the two bee species. However, these differences in concentrations between honeybee and bumblebee pollen must be tempered by the fact that the bumblebee nests and the honeybee hives were placed in different rural areas and by the fact that honeybee pollen was gathered for 4 days using traps, whereas bumblebees foraged for 4 weeks before the pollen was collected in the nests. Nevertheless, it is likely that the pollen sample collected by bumblebees was gathered in the previous two-three days as they keep low storage levels to avoid theft of honey and pollen by mammals (Heinrich 2004).

Our data show that the pollen of oilseed rape crops is contaminated with a broad range of pesticides, notably spiroxamine, carbendazim, the neonicotinoids thiamethoxam and clothianidin, a range of DMI fungicides and trifloxystrobin. Other fungicides, i.e. boscalid, pyraclostrobin and fluoxastrobin were also present but less frequently detected. Broadly similar cocktails, at generally slightly lower concentrations, were found in hand-collected pollen from wildflowers in arable field margins. It should be noted that this is not an exhaustive list of the pesticides present; in particular we did not screen for pyrethroids because these require an entirely different analytical approach, but these were used on the farms we studied.

Some of the neonicotinoids and fungicides that we have detected in honeybee collected pollen had already been detected in similar pollen samples in other studies, although this is the first study providing data in bee pollen for this mixture of pesticides in UK. It should be noted however that these studies used composite pollen samples (as opposed to pollen from individual species here) and therefore provide less information on the variability of exposure levels. In pollen samples from honey bee colonies in western France, carbendazim and flusilazole were detected at concentrations up to

2595 ng/g and 52 ng/g respectively (as opposed to 120 and 6.1 ng/g respectively in our study) (Lambert et al., 2013). Higher concentrations of thiacloprid, imidacloprid, carbendazim, trifloxystrobin, boscalid, tebuconazole, pyraclostrobin and trifloxystrobin were also observed in honeybee pollen collected in hives from North America (up to 962 ng/g for boscalid) (Mullin et al., 2010) but their frequencies were generally much lower than those detected in this study. Overall, our results and these studies indicate that these mixtures of insecticides and fungicides appear ubiquitous in pollen samples and that even higher concentrations than the ones observed in our study can be encountered.

Honey bees and the bumblebee *Bombus terrestris* are both highly polylectic in their flower visits; both are regular visitors to OSR flowers (Cresswell and Osborne 2004), but both taxa also visit a broad range of wildflowers present in field margins and hedgerows, gardens, and uncropped areas, though the two species exhibit different floral preferences (Wood et al., 2015). We would thus expect both species to be exposed to the chemicals we found in pollen of the crop and wildflowers, and indeed this was the case. It is worth noting that for both species, pollen from hawthorn represents a major part of the collected pollen (up to 87%) and that the pollen from hawthorn collected by honeybees was often contaminated by several pesticides (up to 4) and notably at concentrations up to 33 ng/g for carbendazim.

For pollen collected by honeybees, the major pesticide contaminants were (in declining order of mean concentration) carbendazim, boscalid, spiroxamine, tebuconazole and trifloxystrobin, with small amounts of the neonicotinoids thiacloprid, imidacloprid and thiamethoxam. Overall, the concentrations tend to be lower than in the crop or adjacent wildflowers, likely to be because the bees are also collecting pollen from uncontaminated wildflowers distant from arable fields, diluting the overall concentration returning to the hive. There was a notable reduction in the concentrations of neonicotinoids and fungicides detected in honey bee pollen collected after OSR blooming, presumably because the bees are no longer feeding on treated crops but also perhaps because of ongoing biodegradation and photolysis of pesticide residues in the environment as summer progresses (Bonmatin et al., 2015; Gupta et al., 2008).

Concentrations of pesticides in pollen collected by bumblebees were markedly different to those for pollen collected by honeybees during the OSR bloom (Figure 3). The major contaminants were carbendazim, thiamethoxam and flusilazole. The high levels of thiamethoxam are particularly noteworthy, for this is an insecticide of high toxicity to bees. Experimental studies such as Whitehorn et al. (2012) which describe severe impacts of neonicotinoids on bumblebees have been criticised for using unrealistically high concentrations of pesticide (in this example 6 ng/g of imidacloprid) (Carreck

and Ratnieksi 2014). Our data suggest that real-world exposure may often be much higher than this, for the mean concentration of thiamethoxam in our samples from 5 nests located in farmland was 18 ng/g, and one of the nests located in urban environment showed more than 19 ng/g for imidacloprid. It has also been demonstrated that there are synergies between neonicotinoids and DMI fungicides such as flusilazole (Iwasa et al., 2004; Schmuck et al., 2003), so the presence of both compounds at high concentrations in pollen stores of bumblebees is a cause for concern.

Recently, Ründlof et al. (2015) found that bumblebee colonies were adversely affected by proximity to fields of OSR treated with clothianidin (the major bioactive metabolite of thiamethoxam), but that honeybees showed no significant harm, at least within one season. Our results suggest an explanation for this disparity; bumblebees may simply be exposed to the pesticide more, perhaps because of a greater propensity to collect OSR pollen (i.e. proportion of OSR pollen was 10% in average for honeybees as opposed to 32% in average for bumblebees). It may also be because bumblebees tend to forage over shorter distances compared to honeybees (Knight et al., 2005), which may mean that there is less dilution of pesticide residues coming in to the nest when these are located in the vicinity of arable lands. However, it should be noted that our data set is small, and that honeybee hives and bumblebee colonies were not placed in exactly the same localities. They were also sampled in different ways; honeybee pollen was collected from returning bees using a pollen trap, whereas pollen traps are not effective for bumblebees and hence pollen was taken from stores inside the nest. Further research is clearly needed to confirm whether bumblebees really are more prone to collect pollen contaminated with pesticides, and if so, why.

Our sampling was conducted in the spring and summer of 2013. Since then, a moratorium on the use of neonicotinoids as seed dressings on flowering crops has come into effect in the EU (though some individual countries have granted derogations for continued use). It would be fascinating to repeat our work to examine whether contamination of wildflowers and bee pollen with neonicotinoids has dropped as a result.

In contrast to rural areas, there were generally few pesticide residues in pollen collected by bumblebee colonies in the 3 nests placed in urban areas. Imidacloprid was the biggest contaminant, and the only neonicotinoid detected. To our knowledge, these are the first data pertaining to exposure of bees to pesticides in urban environments, and a more extensive study is needed to determine whether pesticide exposures are much lower in these areas. While pesticide usage data in the UK is available for farmland, no data are publicly available on sales or usage of pesticides by gardeners and local authorities, and very little information is available on likely levels of contamination of ornamental plants with pesticides, so we can only speculate as to the source of this exposure. Imidacloprid was

widely sold in the UK as a garden insecticide in the past, but has been largely replaced by thiacloprid and acetamiprid in recent years (D.G. pers. obs.). It is unclear whether the imidacloprid found in our samples is due to persistent residues from past use, or due to ongoing environmental contamination from other sources – for example imidacloprid is the active ingredient in formulations widely used for ant control (e.g. “Maxforce Quantum”, Bayer Crop Science) and for flea control on domestic animals (e.g. “Advantage”, Bayer Crop Science).

It has previously been found that bumblebee populations in gardens are higher than those in farmland (Goulson et al., 2010; Osborne et al., 2008), and our results may in part explain why – because they could be exposed to fewer pesticides. However, they also probably have access to a greater abundance and diversity of floral resources in gardens, and without further experimental manipulations we cannot determine which of these factors is most important.

Screening of whole bees for pesticides detected generally low concentrations, compared to pollen samples (Table 3), although a range of DMI fungicides were found at concentrations exceeding 1 ng/g in some samples, and carbendazim was found at a mean concentration of 4.6 ng/g in bumblebees from rural areas. There were also detectable traces of the neonicotinoids thiamethoxam, acetamiprid and thiacloprid in some bees. For practical reasons, bumblebee pollen and bumblebee individuals were collected at different times (individuals were collected 6 weeks after the pollen was collected, i.e. after the OSR bloom) and this could partially explain the lower concentrations observed for some pesticides in bumblebees. Despite this, it seems likely that pesticides are metabolised at varying rates once consumed by bees; for instance, it has been shown that bumblebees can clear imidacloprid from their body after 2 days of exposure (Cresswell et al., 2014) and a half-life of 5 hours has been recorded for honey bees (Suchail et al., 2004). A recent study has revealed that detoxification of the xenobiotic, nicotine, in bees, was associated with increased energetic investment and antioxidant and heat shock response (du Rand et al., 2015). The process of detoxifying an array of xenobiotics arising from exposure to agrochemicals and secondary plant products may result in metabolic stress and increased susceptibility of the bee to pathogens and disease (Goulson et al., 2015).

It is notable that the bulk of pesticides found in both honeybee pollen and bumblebee pollen were fungicides, particularly carbendazim, boscalid, tebuconazole, flusilazole, metaconazole, pyraclostrobin and trifloxystrobin. Although fungicides have generally low toxicity to bees (Johnson 2015), little is understood about the impacts they may have on beneficial fungi commonly found in stored pollen (bee bread). Classes of fungicides commonly found in bee pollen in our study (boscalid, DMIs and quinone outside inhibitors, QoIs) have been reported to be fungicidal against 12 fungal species isolated from bee bread (Yoder et al., 2012). Bee bread is produced by fungal fermentation of

stored pollen and is important food for honey bee larvae. Alterations in the diversity of fungi may affect food value and also allow pathogenic fungi such as the etiological agent of chalkbrood disease, *Ascosphaera apis*, to thrive in the hive, thus affecting colony performance (Yoder et al., 2013).

In summary, our study confirms that bees foraging in arable farmland are exposed to a complex cocktail of neonicotinoid insecticides and fungicides in the pollen they collect, with exposure of bumblebee colonies being far higher than that of honeybees. While quantifying realistic levels of exposure via pollen as we have done here is an important step forwards, we did not examine exposure via nectar, which we intend to address in future work. A major challenge which has yet to be tackled is attempting to understand what effects simultaneous exposure to multiple pesticides has upon bees in the field.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

Statement on animal ethical care

The work reported here conforms to the regulatory requirements for animal experimentation in the UK. No ethics approval was required for this study. Honeybee hives and bumblebee nests were housed on private land for which research permission was granted by the owners. This study did not involve endangered or protected species.

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